After Final Office Action of January 2, 2008

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method of measuring the amount of a 25-hydroxy vitamin D metabolite, 1α,25-dihydroxy vitamin D metabolite or both in a sample using a competitive protein binding assay, wherein displacement of a vitamin D derivative from a vitamin D binding protein is measured and the vitamin D derivative displaces a 25-hydroxy- or lα,25dihydroxy vitamin D metabolite or both from the vitamin D binding protein,

wherein a displacement efficiency of approximately 1 is obtained by using a vitamin D derivative of formula (I):

wherein:

- represents a 25-hydroxylated side-group of vitamin D₂ or of vitamin D₃; R
- Y represents hydrogen or hydroxy;
- represents a functional group, coupled via a spacer group, selected from the group consisting of biotin, digoxigenin, amino acids, characteristic amino acids and

peptide sequences, FITC, proteins, peptide groups, protein-A, protein G and vitamin D derivatives; and wherein the measurement of displacement of a vitamin D derivative from a vitamin D binding protein in the sample is correlated to the measurement of displacement of a vitamin D derivative from a vitamin D binding protein using a known quantity of the vitamin D derivative to determine the amount of a 25-hydroxy vitamin D metabolite, $1\alpha,25$ -dihydroxy vitamin D metabolite or both in the sample

obtained by a method comprising:

- a) cyanoethylating the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;
- b) adding lithium hydride and converting the 25 hydroxy group into the lithium alcoholate and subsequently reducing the nitrile group with lithium aluminium hydride; and

blinking a spacer group selected from amino carboxylic acid radical and amino polyether radical, together with a functional group A on the resulting amino propylether side chain.

- 2 (**Original**) The method of claim 1, wherein the method is a competitive immunoassay, selected from the group consisting of radioimmunoassay, enzyme immunoassay enzyme-linked immunosorbent assay, luminescence immunoassay and fluorescence immunoassay.
- 3. (**Original**) The method of claim 1, wherein the method is sandwich immunoassay, selected from the group consisting of immuno radiometric assay, IEMA/EIA, immuno luminometric assay and immunofluorometric assay.
- 4 (Currently Amended) A kit for detection of 25-hydroxy- and or 1α/25- dihydroxy vitamin D metabolites or both in a sample on basis of a competitive protein binding assay, wherein displacement of a vitamin D derivative of the formula (I) from a vitamin D binding protein is measured and the vitamin D derivative displaces a 25-hydroxy- or lα,25-dihydroxy vitamin D metabolite from the vitamin D binding protein, comprising a standardized quantity of solid

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vitamin D derivative of formula (I) or a standardized solution of a vitamin D derivative of formula (I):

wherein:

- $R \qquad \text{represents a 25-hydroxylated side-group of vitamin D_2 or of vitamin D_3;} \\$
- Y represents hydrogen or hydroxy;

A represents a functional group, coupled via a spacer group, selected from the group consisting of biotin, digoxigenin, amino acids, characteristic amino acids, peptide sequences,

FITC, proteins, peptide groups, protein-A, protein G and vitamin D derivatives, which can be bound by a protein with high affinity;

wherein the vitamin D derivative is obtained by a method comprising:

- eyanoethylating the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;
- adding lithium hydride and converting the 25 hydroxy group into the lithium alcoholate and subsequently reducing the nitrile group with lithium aluminium hydride; and

e)linking a spacing group, selected from amino carboxylic acid radical and amino polyether radical, together with a functional group A on the resulting amino propylether side chain.

5-6. (Cancelled)

- 7. (Original) The kit of claim 4 comprising a solid phase selected from the group consisting of a microtitration plate, another solid carrier, a microparticle, a polymeric material, and a cellulose.
- 8. (Original) The kit of claim 7, in which the solid phase is a microparticle comprising agarose.
- 9. (Original) The kit of claim 7, in which the solid phase is a magnetic microparticle.

10. (Canceled)

11. (Previously Presented) The method of claim 1, wherein said competitive protein binding assay is selected from the group consisting of an enzyme immunoassay, an enzyme-linked immunosorbent assay, a radio immunoassay, an immunoradiometric assay, a luminescence assay, a fluorescence immunoassay and an immunofluorometric assay.

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- 12. (New) The method of claim 1 wherein Y is hydroxy.
- 13. (New) The kit of claim 4 wherein Y is hydroxy.